A QUANTITATIVE STUDY ON FEEDBACK CONTROL OF LH BY TESTOSTERONE IN YOUNG ADULT AND OLD MALE RATS

By Karl M. Pirke, Michael Geiss and Rainer Sintermann

ABSTRACT

The hypothalamic-pituitary gonadal axis was studied in young adult (3 month old) and old (24 to 27 month old) male Wistar rats. Plasma testosterone decreased significantly in old animals (x: 262 ng/100 ml (n = 35); versus x: 110 ng/100 ml (n = 30)). The fall in LH was less pronounced but still significant (54.5 ng LH-RP-1/ml in young versus 39.5 ng/ml in old rats). Groups of 6 to 8 animals of both ages were castrated and implanted with silastic capsules continuously releasing testosterone. The length of the capsules was directly proportional to the plasma testosterone levels achieved (range between 63 and 350 ng/100 ml).

After one week young castrated rats not substituted with testosterone showed LH values three times higher (x: 351 ng/ml) than old rats treated in the same way (x = 126 ng/ml). LH values in the animals substituted with testosterone indicate that the sensitivity of the negative testosterone-LH feedback is greatly increased in old rats. Testosterone can be depressed to 60 ng/100 ml before an increase in LH occurs. In young rats no increase in LH was observed when testosterone values were higher than 170 ng/100 ml. In the range between 170 and 100 ng/100 ml about half of the young animals reacted with increased LH secretion, while an increase was observed in all young animals when testosterone dropped below 100 ng/100 ml.

A decrease in incretory testicular function is observed in many mammalian species, including man (for review see Leathem 1977). In the rat, a significant fall in plasma testosterone is observed when animals reach an age of about 2 years (Ghanadian et al. 1975; Chan et al. 1977; Pirke et al. 1978). Since the
in vivo (Harman et al. 1978) and the in vitro responses (Pirke et al. 1978) of
the testes to HCG stimulation is unchanged in the old rat and since the plasma
LH concentrations decrease with age (Shaar et al. 1975; Riegle & Meites 1976),
it can be assumed that the age-dependent decrease in Leydig cell function is
mainly caused by an impaired hypothalamic-pituitary function.

In this study we have tried to elucidate further the nature of the hypo-
thalamic-pituitary dysfunction by performing a quantitative study on the nega-
tive testosterone-LH feedback in young adult and old male Wistar rats. We
have manipulated plasma testosterone levels by castrating the animals and im-
planting in them silastic capsules, which continuously release testosterone
(Legan et al. 1975; Damassa et al. 1976). We thereby established a constant
feedback signal and eliminated the rapid fluctuations of plasma testosterone
observed in the intact male rat (Barke et al. 1973). In this way we obtained
data on the sensitivity and on the strength of the LH response in the gonadal
hypothalamic-pituitary system in young adult and old male rats.

M A T E R I A L S A N D M E T H O D S

Materials

Testosterone was obtained from Merck AG, Darmstadt. Silastic tubes (inner diameter
2 mm, outer diameter 3 mm) were supplied by Deutsch & Neumann, Berlin. Controlled
release capsules were prepared by filling the tube tightly with testosterone. Both ends
of the tube were closed with stainless steel plugs. The capsules were incubated for 24 h
in 0.01 M phosphate buffered saline and then sterilized. The capsule sizes, representing
the length of tubing filled with testosterone, were 0.5, 1.0, 1.5 and 3.0 cm. The reagents
for the radioimmunoassay of rat LH were a gift of the NIAMDD Rat Pituitary Dis-
tribution Program.

Methods

Testosterone was measured by a radioimmunoassay, including thin-layer chromato-
graphy, as described earlier (Pirke 1973). The inter-assay variability was 8.9% (CV) at
an average concentration of 424 ng/100 ml. Rat LH was measured according to the
recommendations of the Rat Pituitary Hormone Distribution Program. The precision
was 11.5% (CV) at a concentration of 42.4 ng LH-RP-1/ml.

Animals

Young adult (3 month old) and old (24 to 27 month old) male Wistar rats were
obtained from the Central Institute for Laboratory Animals, Hannover. The animals
were kept under a 16-h light, 8-h dark schedule. Four (old) or 6 (young) animals were
kept in a cage. Water and Altrumin® rat food were available ad libitum.

Experiments

Thirty-five young adult and 35 old rats were divided into groups of 6 to 8 animals.
Transcrotal castration was performed under anaesthesia. Silastic tubes of 0.5, 1.0,
1.5 and 3.0 cm length were implanted subcutaneously in the back of the animals im-
mediately after castration. An intraperitoneal injection of penicillin was administered after the operation. After one week the animals were killed by decapitation. The trunk blood was collected, centrifuged and the serum stored at -30°C until analysed. Great care was taken to kill the animals immediately after removal from their cages in order to avoid stress-related changes in LH. All animals were killed between 1 and 2 p.m.

RESULTS

The concentration of testosterone and LH was measured in 35 young adult (3 months) and in 30 old (24 to 27 months) untreated male rats. The LH data for both age groups are given in Table 1. LH was significantly lower in the old than in the young animals. The testosterone values in these control groups ranged from 72 to 1162 ng/100 ml (\(\bar{x} = 262\) ng/100 ml) in the young group and from 23 to 263 ng/100 ml (\(\bar{x} = 110\) ng/ml) in the old group. The age-dependent fall in testosterone was thus more pronounced than the fall in LH values.

The testosterone values achieved by implantation of silastic capsules which release testosterone continuously are given in Fig. 1. A linear relationship exists

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Plasma LH in young adult and old castrated rats implanted with testosterone-releasing capsules of different size.</th>
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<tbody>
<tr>
<td></td>
<td>Young adult rats</td>
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<tr>
<td></td>
<td>x ± sd range ng LH-RP-1/ml</td>
</tr>
<tr>
<td></td>
<td>x ± sd range ng LH-RP-1/ml</td>
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<tr>
<td>Controls</td>
<td>55 ± 16 26-95</td>
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<tr>
<td>Castrated</td>
<td>351 ± 150 195-600</td>
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<tr>
<td>Castrated</td>
<td>283 ± 169 63-500</td>
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<tr>
<td>Castrated</td>
<td>176 ± 246 25-665</td>
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<td>Castrated</td>
<td>47 ± 28 18-91</td>
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<td>22 ± 10 8-35</td>
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between capsule length and plasma testosterone in the castrated young and old animals. Plasma concentrations in the old rats were smaller than in the young rats, at least when the capsules of 1.0, 1.5 and 3.0 cm length are considered. This difference is due to the fact that the body weight was significantly ($P < 0.01$) greater in the old rats. The average weight of the young animals was 268 g (range = 215 to 330 g) and 463 g (range = 360 to 610 g) in the old rats. The difference in body weight is thus much more pronounced than the difference in the testosterone concentrations achieved.

The plasma LH values obtained in castrated animals with or without testosterone implants are given in Table 1. LH values were not different for young and old animals implanted with capsules of 1.5 and 3.0 cm length. Significantly higher LH values were observed in young rats implanted with capsules of either 1.0 or 0.5 cm length. The LH levels were almost three times greater in the young than in the old castrated animals without implantation. The LH levels in the old rats remain low even with the smallest implants. Young animals implanted with capsules of 1.0 and 0.5 cm length revealed elevated LH concentrations.

In Fig. 2 the plasma testosterone levels achieved are plotted against the LH values. The solid horizontal line indicates the upper limit of the normal range.

![Fig. 1](image-url)

**Fig. 1.**
Plasma testosterone as a function of testosterone capsule length in young adult (●) and old (○) castrated male Wistar rats.

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Plasma testosterone and LH after castration and implantation of testosterone-releasing capsules in young adult (●) and old (○) male Wistar rats. The solid horizontal line indicates the upper limit of the normal range for the young rats, and the dotted horizontal line indicates the upper limit for the old rats. For further explanation, see text.

Fig. 2.

DISCUSSION

When adult male rats are orchiectomized, a rapid increase in LH in plasma occurs. Serum concentrations reach a plateau after 24 h and then remain constant for a week. Thereafter a further increase in plasma LH is observed (Yamamoto et al. 1970; Badger et al. 1978). The increase in LH reported here represents the initial response of the hypothalamic pituitary system to the re-
moval of testosterone. Our data confirm the observations of Riegle et al. (1977), who observed significantly higher LH values after castration in young adult rats.

The release of testosterone from silastic capsules at a constant rate over a period of several weeks has been described by Damassa et al. (1976). This technique offers great advantages compared to the injection of testosterone or testosterone esters, since the hypothalamic pituitary response can be related to a constant level of circulating testosterone. Damassa et al. (1976) used constantly-releasing capsules to study the testosterone LH feedback in adult male Long-Evans rats. They were able to discriminate between two different populations. About three-quarters of the animals displayed an LH increase, when testosterone concentrations in plasma dropped below 110 ng/100 ml, while the rest did not show elevated LH values until the testosterone values were below 60 ng/100 ml. We also observed individual differences in the sensitivity of the feedback in young adult male Wistar rats. The sensitivity of the feedback seems, however, to be somewhat smaller in the adult Wistar rat than in Long-Evans rats, since all young adult animals studied have displayed elevated LH values when testosterone dropped below 100 ng/100 ml and about half of the rats (3 out of 7) showed an increase in gonadotrophin secretion in the range between 100 and 170 ng testosterone/100 ml. In contrast to the young adult rats, an increase in plasma LH was observed in the old rats only when testosterone values dropped below 60 ng/100 ml. This finding indicates that the sensitivity of the testosterone-LH feedback is increased in the old animals. Shaar et al. (1973) injected young and old male rats after castration with different doses of testosterone propionate. They observed that a smaller dose of the androgen per body weight is necessary to suppress LH in the old males. This finding could, however, be explained by a smaller metabolic clearance rate of testosterone in the old animals. That testosterone may indeed be metabolized at a slower rate in old rats is indicated by our observation that testosterone implants of the same size bring about only slightly lower testosterone levels in the plasma of the old rats, despite the fact that the body weight of the old animals was much greater than that of the young rats (463 g versus 268 g).

In the group of untreated young rats 2 animals were observed which had plasma testosterone values below 100 ng and 8 animals with testosterone values below 170 ng. In the group of old untreated rats, 4 had plasma testosterone levels below 60 ng/100 ml. None of these animals revealed high LH concentrations. This observation, which seems to be in contradiction to the results of the castration and substitution study reported here, can probably be explained by the fact that testosterone concentrations fluctuate rapidly and to a great extent in the rat (Bartke et al. 1973). The testosterone values in the untreated animals are therefore most likely not in all cases representative for the average blood concentrations.
In the hypothalamus testosterone can be metabolized to dihydrotestosterone and to oestradiol. The feedback effect of testosterone may be mediated by one or both of these metabolites. An increased sensitivity of the testosterone-LH feedback could therefore be explained by an increased conversion of testosterone to dihydrotestosterone or oestradiol. Future studies on the age-dependence of hypothalamic testosterone metabolism may therefore give some insight into the mechanisms of altered feedback sensitivity in the aging rat.

In conclusion, our study indicates that the hypothalamic-pituitary system in the old rat is able to increase LH secretion when adequately stimulated, although the increase after castration is smaller than in young adult rats. Since, on the other hand, the response of the testes to HCG stimulation in vitro and in vitro (Harman et al. 1978; Pirke et al. 1978) is unchanged in the old rat, it can be concluded that the increased sensitivity of the testosterone-LH feedback in the old rat is responsible for the decreased Leydig cell function in old age.

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REFERENCES


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